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Wound-Induced Expression of the *FAD7* Gene Is Mediated by Different Regulatory Domains of Its Promoter in Leaves/Stems and Roots[1]

Takumi Nishiuchi[2], Hiroaki Kodama[3], Shuichi Yanagisawa, and Koh Iba*

Department of Biology, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812–8581, Japan (T.N., H.K., K.I.); and Department of Life Sciences (Chemistry), Graduate School of Arts and Sciences, University of Tokyo, Meguro, Tokyo 153–8902, Japan (S.Y.)

The *FAD7* gene is expressed preferentially in the chlorophyllous tissues of unwounded plants. Wounding activates the expression of the *FAD7* gene not only in chlorophyllous tissues, but also in nonchlorophyllous tissues of stems and roots. Our previous study suggested that wound-responsive transcriptional activation by the *FAD7* promoter in leaves/stems and roots is brought about by a jasmonic acid (JA)-independent and JA-dependent signaling pathway, respectively. In this paper, we show that a specific region (from −259 to −198) in the *FAD7* promoter is required for wound-activated expression of this gene in leaves and stems, while another region (from −521 to −363) is necessary not only for wound-activated but also for JA-responsive expression of this gene in roots. Thus, different regulatory regions of the *FAD7* promoter mediate distinct wound-induced expression of this gene in leaves/stems and roots. Gel mobility shift assays revealed the wound-inducible DNA-binding activity to the −242/−223 region in both stem and leaf nuclear extracts. In fact, deletion of this region abolished wound response of the *FAD7* promoter, suggesting the in vivo role of this site. Furthermore, we detected root nuclear factors interacting with the region from −433 to −363 of this promoter. Wounding and methyl jasmonate treatments induced differently these DNA-binding activities. These results suggest that different regulatory mechanisms mediate the wound-induced expression of the *FAD7* gene in aerial and subterranean organs.

Plants respond to wounding by activating a set of defensive genes, such as proteinase inhibitor II (*pinII*), and most of these play some role in wound healing and the prevention of subsequent pathogen invasion (Bowles, 1990). Jasmonic acid (JA), a fatty acid-derived hormone, is one of several candidate molecules for wound signaling and is thought to play a pivotal role in the transcriptional activation of wound-inducible genes (Farmer and Ryan, 1992; Farmer et al., 1998). Wounding activates the octadecanoid pathway in which linolenic acid is converted to JA, resulting in a significant accumulation of this hormone. The elevated JA level is thought to cause transcriptional activation of many wound-responsive genes (Creelman et al., 1992; Peña-Cortés et al., 1993).

On the other hand, recent findings have suggested the involvement of a JA-independent signal transduction pathway in wound-induced gene expression. Wound-responsive expression of the glutathione *S*-transferase (*GST*) gene was observed in the leaves of JA-deficient Arabidopsis *fad3 fad7 fad8* triple mutants (McConn et al., 1997). In addition, the expression of choline kinase (*CK*) and wound-responsive 3 (*WR3*) genes, which were first isolated as wound-inducible genes in Arabidopsis leaves by the differential display technique, was induced by wounding even in the JA-insensitive *coi1* Arabidopsis mutants (Titarenko et al., 1997). Furthermore, exogenous application of JA failed to induce the expression of several wound-responsive genes, such as the genes encoding tobacco ethylene-responsive transcription factors (ERFs) and tomato glucosyl transferase (O'Donnell et al., 1998; Suzuki et al., 1998).

Membrane-associated ω-3 fatty acid desaturases catalyze the desaturation of dienoic fatty acids (18:2+16:2) to trienoic fatty acids (18:3+16:3), which is the starting material for the biosynthesis of fatty acid-derived signaling molecules such as JA (Farmer, 1994; Weber et al., 1997; Farmer et al., 1998). Higher plants contain the plastidial ω-3 fatty acid desaturase (FAD7 enzyme) and the microsomal ω-3 fatty acid desaturase (FAD3 enzyme). The mRNA of the *FAD3* gene was present in both leaves and roots of Arabidopsis (Yadav et al., 1993) and tobacco (Hamada et al., 1994). On the other hand, the transcript of the *FAD7* gene was observed only in the chlorophyllous tissues of Arabidopsis (Nishiuchi et al., 1995) and tobacco (Hamada et al., 1996). Wounding treatments increased the amount of *FAD7* mRNA but not the amount of *FAD3* mRNA in tobacco leaves (Hamada et al., 1996). The *FAD7* gene was activated by wounding similarly in the leaves and roots of Arabidopsis plants (Nishiuchi et al., 1997). Thus, it is likely that the *FAD7* genes play an important role in the wound

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[2] Present address: Plant Molecular Biology Laboratory, Molecular Biology Department, National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, 1–1 Higashi, Tsukuba 305–8566, Japan.

[3] Present address: Department of Bioresources Chemistry, Faculty of Horticulture, Chiba University, Matsudo 648, Chiba 271–8510, Japan.

* Corresponding author; e-mail koibascb@mbox.nc.kyushu-u.ac.jp; fax 81–92–642–2621.

response of higher plants by supplying a precursor for JA biosynthesis (Nishiuchi and Iba, 1998).

In our previous study, wounding activated the Arabidopsis *FAD7* gene not only in chlorophyllous tissues but also in nonchlorophyllous tissues, resulting in drastic changes of its spatial expression pattern (Nishiuchi et al., 1997). Studies of the exogenous application of JA and inhibitors for JA biosynthesis suggested that wound-induced expression of the *FAD7* gene in roots depended on JA biosynthesis, whereas wound-induced expression in leaves and stems did not depend on JA biosynthesis (Nishiuchi et al., 1997). Thus, the Arabidopsis *FAD7* promoter provides a unique model for studying the mechanism of transcriptional activation in response to wounding through different signal transduction pathways. In this paper, we mapped the wound-responsive regions of the *FAD7* promoter in each vegetative organ. Moreover, we detected the tobacco nuclear factors that interact with the wound-responsive regions of the *FAD7* promoter in response to wounding.

MATERIALS AND METHODS

Construction of the *FAD7* Promoter-β-Glucuronidase (*GUS*) Fusions and Generation of Transgenic Tobacco Plants

Construction of fD82, fD52, fD36, fD16, and fD7 has been described previously (Nishiuchi et al., 1995). These constructs carried a *GUS* reporter gene under the control of the Arabidopsis *FAD7* promoters truncated at −825, −521, −362, −165, or −76.

Chimeric constructs were introduced into tobacco (*Nicotiana tabacum* cv W38) plants using the leaf disc method. The R1 seeds of fD82, fD52, fD36, fD16, and fD7 transformants were aseptically germinated in Murashige-Skoog medium containing 100 μg/mL kanamycin. The kanamycin-resistant R1 seedlings were then transferred to soil, grown at 26°C under continuous fluorescent illumination (2,000 lux), and subjected to further analysis of their wound responsiveness. Wounding treatments of each organ were performed as previously described (Nishiuchi et al., 1997).

Fluorometric GUS Assay

GUS activities were fluorometrically determined in both unwounded and wounded samples from each organ of these transgenic tobacco plants, as previously described (Nishiuchi et al., 1997). The ratio of GUS activity in wounded tissues to that in unwounded tissues was expressed as wound responsiveness. Plants harboring a series of 5′-deleted *FAD7* promoter-*GUS* fusion genes were hydroponically fed with 100 μM MeJA solutions for 6 h, as previously described (Nishiuchi et al., 1997), and the promoter activity was fluorometrically determined.

Gel Mobility Shift Assay

Nuclear extracts were prepared from wild-type plants that were grown in a greenhouse as described by Green et al. (1989). Tissue in aerial parts was cut off and immediately transferred into liquid N_2. Alternatively, root tissue was carefully washed to remove the soil and then frozen by liquid N_2. Each tissue sample was subjected to the extrac-

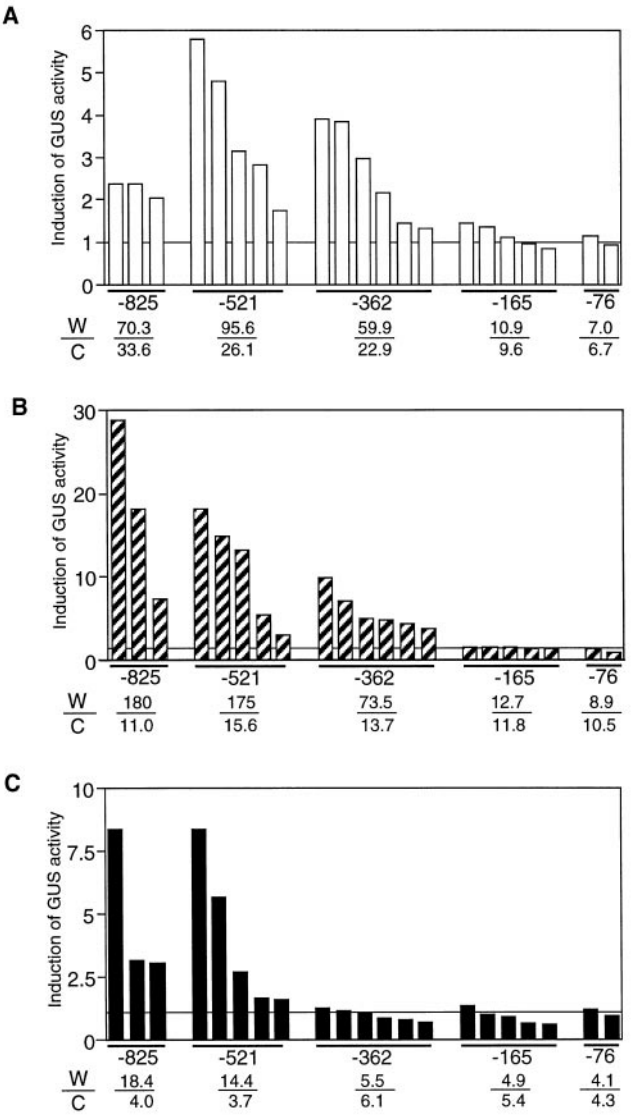

Figure 1. 5′-Deletion analysis of the *FAD7* promoter concerning wound responsiveness in each organ. Tobacco plants carrying a *GUS* gene under the control of derivatives of the *FAD7* promoter were grown on soil at 26°C under continuous illumination for about 3 months. Wounding treatments were carried out as described in "Materials and Methods." GUS activities ($n = 5$) were determined in both unwounded and wounded young portions of leaves (A), stems (B), and roots (C) in each *FAD7* promoter-*GUS* transgenic line. For each construct, two to six independent transgenic lines were investigated. C and W indicate, respectively, average values of GUS activities (nmol methylumbelliferone min^{-1} mg^{-1} protein) in unwounded and wounded tissues of the transgenic lines examined. Induction of GUS activity by wounding is expressed by the ratio of the average GUS activity in wounded tissues to that in unwounded tissues. Each bar indicates the GUS activity of an individual transgenic line. The horizontal line represents the position where the value of the ratio is 1.0.

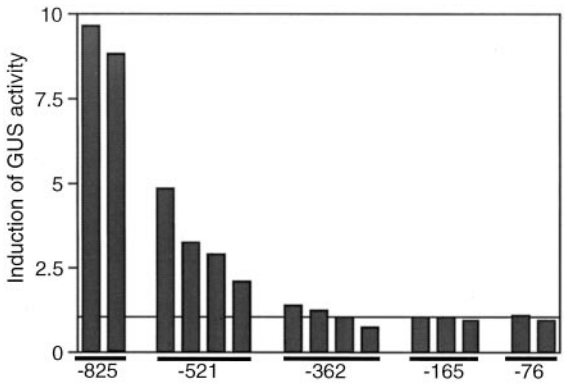

Figure 2. 5′-Deletion analysis of the *FAD7* promoter concerning MeJA responsiveness in roots. As described in "Materials and Methods," MeJA solution was hydroponically applied to tobacco plants carrying a series of the 5′-deleted *FAD7* promoter-*GUS* fusion genes that had been grown for about 3 months. GUS activities ($n = 5$) were determined in both untreated and MeJA-treated roots in each *FAD7* promoter-*GUS* transgenic line. Induction of GUS activity by MeJA application is expressed by the ratio of the average GUS activity in MeJA-treated tissues to that in untreated tissues. Each bar represents an individual transgenic line. The horizontal line represents the position where the value of the ratio is 1.0.

tion of the nuclear protein as "unwounded" samples. Cut sections of leaves, stems, and roots were incubated in sodium phosphate buffer for 2, 2, and 4 h, respectively, and then subjected to the preparation of nuclear proteins as "wounded" samples. In addition, nuclear proteins were extracted from the roots of plants that were hydroponically fed with 100 μM MeJA solutions for 4 h. DNA fragments were labeled at the 5′ end with [γ-^{32}P]ATP using T4 polynucleotide kinase. The DNA-binding reactions were carried out in 25 mM HEPES/KOH (pH 7.5), 100 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 10% (v/v) glycerol, 10,000 cpm radioactively labeled DNA, and 1 μg of poly(dI-dC). Nuclear extracts containing 5, 10, and 10 μg of protein prepared from leaves, stems, and roots, respectively, were added to the reaction mix. The competitor DNA was added at the concentration noted in the figures. After 10 min of incubation at room temperature, samples were electrophoresed on a 4% (w/v) polyacrylamide gel in 0.5× Tris-acetate-EDTA buffer at 4°C. Gels were dried and autoradiographed.

RESULTS

Different Regions of the *FAD7* Promoter Mediate Distinct Wound-Induced Expression in Leaves/Stems and Roots

To identify the wound-responsive region of the *FAD7* promoter, we analyzed the wound-responsive expression of the *GUS* reporter gene, which was fused to several unidirectional 5′-deleted *FAD7* promoters (Nishiuchi et al., 1995). Previous reports have demonstrated an approximately 2-fold induction of GUS activity by wounding in the leaves of three independent −825 *FAD7* promoter-*GUS* transformants (Nishiuchi et al., 1997). In addition, the −521 and the −362 *FAD7* promoter fragments also conferred the characteristic of wound-induced expression in leaves (Fig. 1A). However, a further deletion to −165 in the promoter lead to a failure in the induction of GUS activity by wounding. These results indicated that the promoter region from −362 to −166 contributes to the wound-responsive expression of the *FAD7* gene in leaves.

In stems of transgenic tobacco plants, the −825 *FAD7* promoter fragment directed wound-activated *GUS* expression (Fig. 1B; Nishiuchi et al., 1997). When this promoter fragment was deleted stepwise to −362, the degree of wound induction by this promoter gradually decreased, although induction by wounding was still evident in the transgenic plants harboring the −362 promoter construct. Deletion to −165 caused a complete loss of wound responsiveness in stems, suggesting that the region between −362 and −166 is important for wound activation mediated by the *FAD7* promoter in stems as well as leaves.

Wound induction was also observed in roots of three independent −825 *FAD7* promoter-*GUS* transformants (Fig. 1C; Nishiuchi et al., 1997). Although wounding substantially activated *GUS* expression in the promoter deleted to −521, the promoter with a further deletion to −362 failed to direct the wound-responsive expression of the *GUS* gene in roots (Fig. 1C). Therefore, the wound-responsive region of the *FAD7* promoter in roots, which was localized to the region from −521 to −362, was distinct from the region in stems and leaves.

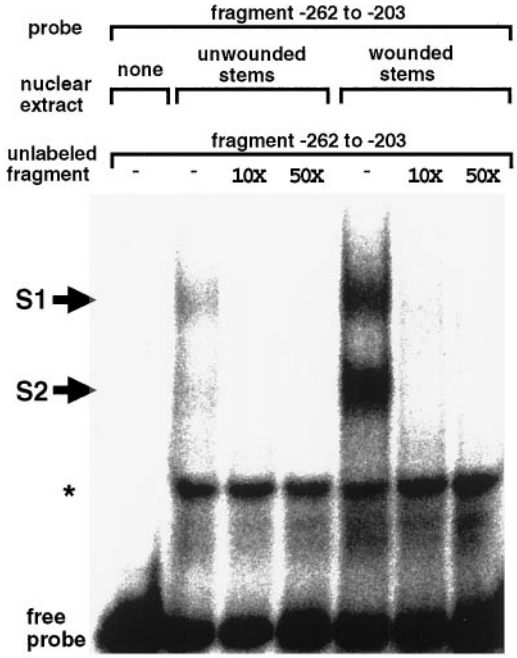

Figure 3. A wound-inducible nuclear factor in stems binds to the −262 to −203 fragment of the *FAD7* promoter. The labeled DNA probe was incubated with or without 10 μg of tobacco nuclear proteins from unwounded or wounded stems. The unlabeled −262/−203 fragment was added as a competitor DNA to the binding reaction mixture at 10- and 50-fold molar excesses. The specific complexes S1 and S2 are indicated by arrows. The position of nonspecific bands is marked by an asterisk.

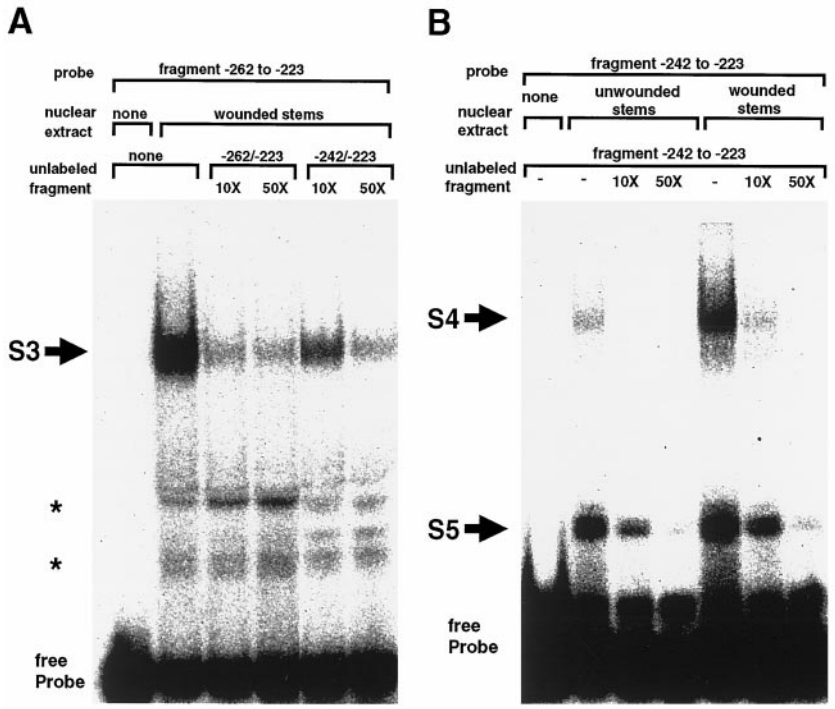

Figure 4. Identification of the binding site of a wound-inducible nuclear factor in stems. A, Competitive binding assays using the −262 and −223 radiolabeled probes of the *FAD7* promoter. An unlabeled 20-bp fragment (−242/−223) was added to the binding reaction at at 10- or 50-fold molar excesses before the addition of 10 μg of tobacco nuclear protein from wounded leaves. The specific complex S3 is indicated by an arrow. The position of nonspecific bands is marked by an asterisk. B, Binding assays of stem nuclear factor to a labeled −242/−223 fragment of the *FAD7* promoter. The −242/−223 probe was incubated with 10 μg of tobacco nuclear proteins from unwounded or wounded stems. The unlabeled DNA fragment was added to each reaction mixture at 10- or 50-fold molar excesses. The specific complexes S4 and S5 are indicated by arrows.

Previous reports have suggested that wound activation by the *FAD7* promoter in roots requires the accumulation of JA (Nishiuchi et al., 1997). We mapped the JA-responsive region of the *FAD7* promoter by exogenous feeding of methyl jasmonate (MeJA). Removal of the *FAD7* promoter region from −521 to −362 caused a loss of JA responsiveness in roots, suggesting that a specific region (−521 to −363) is important for both wound- and JA-responsive expression in roots (Fig. 2).

A Wound-Activated Nuclear Factor in Stems and Leaves Binds to a 20-bp Fragment (−242/−223 Region) of the *FAD7* Promoter

Analyses using deleted promoters of the *FAD7* gene suggested that a critical *cis*-element for wound response in leaves and stems must be present in the region from −362 to −166. Therefore, further delineation of the wound-responsive region in leaves and stems was carried out using the *FAD7* promoter deleted to −259 or −197. In both stems and leaves, wound responsiveness was completely lost by the removal of the region from −259 to −198, indicating that this region is necessary for the wound responsiveness of the *FAD7* promoter (data not shown).

An initial survey of nuclear factors involved in wound response was carried out by gel mobility shift assays with nuclear extracts from wounded or unwounded stems of tobacco, since wounding increased the activity of the *FAD7* promoter more drastically in the stem than in the leaf (Nishiuchi et al., 1997; Fig. 1). As shown in Figure 3, two specific complexes, S1 and S2, were weakly formed by incubation of the −262/−203 fragment with nuclear proteins prepared from unwounded stems. Enhanced formation of these complexes was clearly observed by use of a nuclear extract from wounded stems, suggesting the presence of a wound-activated factor in stems. The complex S1 was always observed, while formation of the complex S2 depended on the preparation.

To define the binding sites of the wound-activated nuclear factors present in stems, we dissected the −262/−203 fragment into two overlapping 40-bp fragments (−262/−223 and −242/−203 regions). By utilizing these probes, we obtained similar patterns of gel mobility shift to that obtained with the −262/−203 fragment (data not shown), suggesting that wound-activated nuclear factors in stems bind to the overlapping region (−242 to −223) of the two fragments.

Further analysis was performed using three 20-bp fragments (−262/−243, −252/−233, and −242/−223) as unlabeled competitor DNA fragments in a mobility shift assay. When the −262/−223 probe was used, the formation of specific complex S3 that was enhanced by wounding

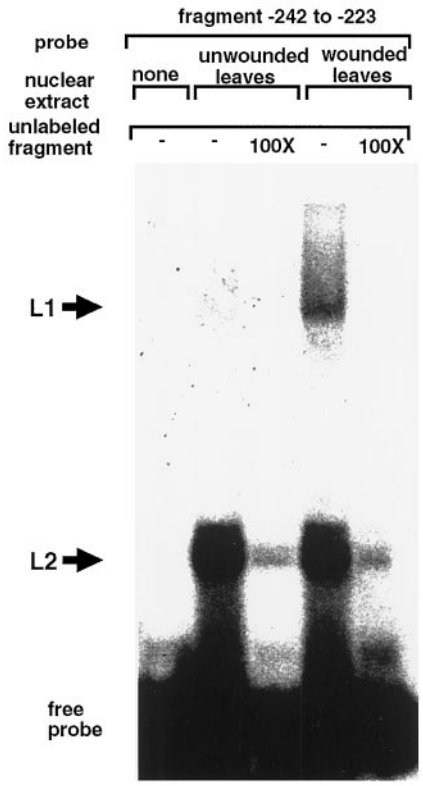

Figure 5. Wound-inducible DNA-binding activity in leaves. The labeled DNA probe (−242 to −223) was incubated with 5 μg of tobacco nuclear protein from unwounded or wounded leaves. Competitor DNAs were added at 100-fold molar excesses of the labeled probe. The specific complexes L1 and L2 are indicated by arrows.

was efficiently inhibited by the −242/−223 fragment (Fig. 4A), but was only slightly inhibited by the −262/−243 fragment (data not shown). The −252/−233 fragment did not interfere with the binding of the wound-activated factors to the probe (data not shown). Furthermore, one specific retarded complex, S4, by a wound-activated nuclear factor was clearly observed when a labeled −242/−223 fragment was incubated (Fig. 4B). Therefore, we concluded that a 20-bp fragment ranging from −242 to −223 contains the binding site of a stem nuclear factor whose DNA-binding activity or amount is enhanced by wounding. The use of the −242/−223 fragment as a probe also provided another specific complex, S5, whose counterpart was not observed when other fragments were used as probes (Fig. 4B). However, the formation of this complex was not modulated by wounding.

We also examined whether the nuclear extract from leaves contained a similar DNA-binding activity to the −242/−223 probes. As shown in Figure 5, a slow-retarded complex, L1, which was not detected with nuclear proteins from unwounded leaves, was observed in the assays with nuclear proteins from wounded leaves. The mobility of the complex L1 was similar to the complex S4 observed with the stem nuclear extracts, suggesting that a common wound-activated nuclear factor may bind to this 20-bp fragment in leaves and stems. Another specific complex, L2, that might correspond to the S5 complex derived from stem extracts, was also observed (Fig. 5). This complex was not responsive to wounding, like the S5 complex.

A 20-bp Segment (−242 to −223) of the *FAD7* Promoter Was Important But Not Sufficient for Wound-Activated Expression in Stems and Leaves

Mobility shift assays suggested that a wound-activated factor binds to a 20-bp segment (−242 to −223) in the *FAD7* promoter in leaves and stems. To determine the function of this region in vivo, tobacco plants were transformed with a construct in which the −242/−223 region was deleted from −259 *FAD7* promoter-*GUS* constructs. These transgenic lines did not exhibit significant wound-induced *GUS* expression (data not shown), suggesting that this 20-bp fragment contributes to wound-responsive expression in stems and leaves. However, a hybrid promoter with a −262/−203 fragment fused to a minimal 35S promoter truncated at −72 was not able to direct the wound-induced gene expression in stems and leaves (data not shown). Thus, this 60-bp fragment alone was insufficient for wound-responsive expression in leaves and stems.

Wounding and MeJA Treatments Differently Modulate the Binding Activities of Nuclear Factors to the −433/−363 Fragment in Roots

To investigate the regulatory mechanism of wound- and JA-responsive expression by the *FAD7* promoter in roots, nuclear proteins were prepared from wounded, MeJA-treated, and untreated roots of soil-grown tobacco plants. The quality of each nuclear extract was verified by gel mobility shift assay using an *as-1* element as a control probe (Jupin and Chua, 1996). Each preparation had a similar ability to form a specific retarded complex with the labeled *as-1* probe (Fig. 6B). The extracts were then assayed for their ability to bind to the −521/−363 region of the *FAD7* promoter. To perform gel mobility shift assays, we divided this region into two fragments (−521/−434 and −433/−363 regions). The −521/−434 probe did not form a specific retarded complex with any of the nuclear extracts described above (data not shown). On the other hand, when the −433/−363 probe was incubated with the nuclear proteins from wounded roots, it formed two specific complexes, R1 and R2 (Fig. 6A). Complex R1 was more clearly observed when the −433/−363 probes were incubated with the nuclear proteins from MeJA-treated roots (Fig. 6A). In contrast, the R2 complex was specifically observed with the nuclear proteins from wounded roots. Taken together, wounding and the MeJA treatments modulated the interaction between nuclear factors and the −433/−363 region of the *FAD7* promoter differently.

Organ Specificity of Each Nuclear Factor

We also examined distribution of nuclear factors identified in this study. When the −262/−203 probes were incubated with nuclear extracts from untreated, wounded, and MeJA-treated roots, only minor but specific binding

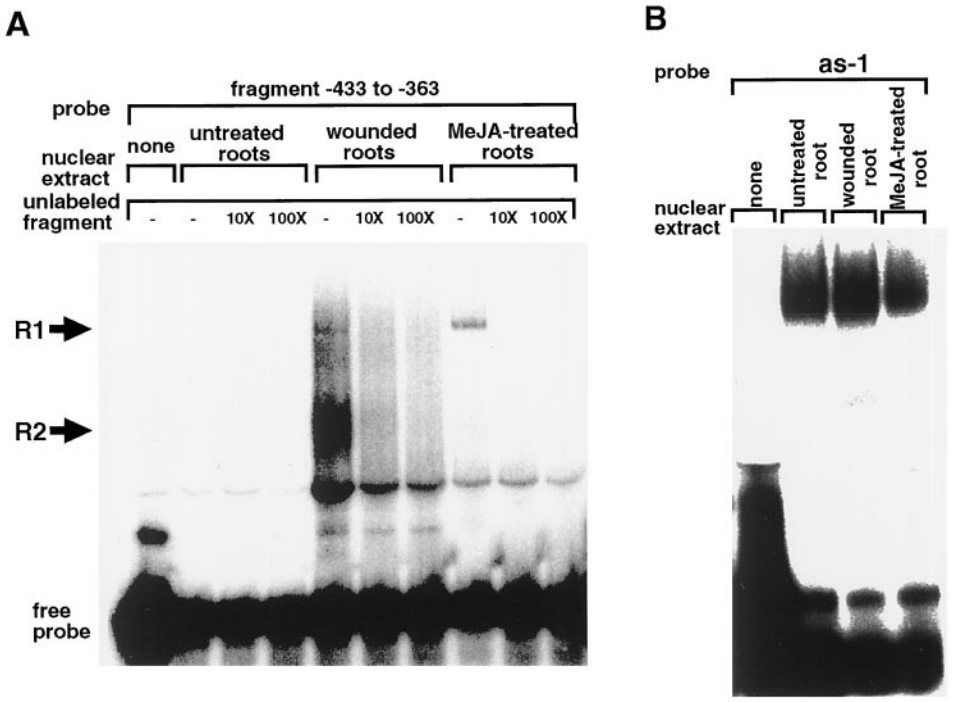

Figure 6. Wounding and MeJA application modulated the DNA-binding activity of root nuclear factors interacting with a region (−433 to −363) of the *FAD7* promoter. The −433/−363 fragment (A) of the *FAD7* promoter and an *as-1* element (B) were used as probes of mobility shift assays. Binding reaction was performed with or without of 10 μg of tobacco nuclear protein from untreated, wounded, and MeJA-treated roots. The unlabeled −433/−363 fragment was added to each binding reaction mixture at 10- and 50-fold molar excesses. The specific complexes R1 and R2 are indicated by arrows.

complexes were observed in all of the nuclear extracts examined (data not shown). Although the mobility of these complexes was similar to that of the complexes observed in stems, we did not observe a difference in the activity of formation of these complexes within each of the nuclear extracts. Thus, it is unlikely that the formation of those complexes is directly involved in the regulatory mechanism of the wound response in roots. In addition, these nuclear factors should play only minor roles for regulation of *FAD7* expression, since deletion of the −362/−166 region (including the −262/−203 region) had no effect on the promoter activity in unwounded roots (Fig. 1).

We examined whether JA- and/or wound-inducible nuclear factors observed in roots are involved in wound-induced expression of the *FAD7* gene in leaves and stems. However, we were unable to detect any specific binding of nuclear proteins in unwounded or wounded leaves or stems by gel mobility shift assay with the −433/−363 probes (data not shown), suggesting that nuclear factors binding to this probe would be specific to wounded root tissues. Therefore, the function of these nuclear factors in roots is specific to the molecular mechanism for wound induction of the *FAD7* gene expression in roots, being quite distinguishable from that in aerial parts.

DISCUSSION

The Arabidopsis *FAD7* gene is expressed preferentially in the chlorophyllous tissues of unwounded plants (Nishiuchi et al., 1995). Wounding drastically changes the spatial expression pattern of the *FAD7* gene in vegetative organs (Nishiuchi et al., 1997). We show that a specific region (from −259 to −197) of the *FAD7* promoter is required for wound-activated expression of this gene in leaves and stems, while another region (from −521 to −363) is necessary not only for wound-responsive but also for JA-responsive expression of this gene in roots (Figs. 1 and 2). These regions appeared to be specifically involved in the tissue-dependent wound response of the *FAD7* promoter but not in the basal activity, because deletion of these regions did not affect the promoter activity in unwounded tissues except leaves (Fig. 1). We also showed that the binding of nuclear factors to these regions was regulated by wounding and JA application.

In this study, we found a wound-inducible nuclear factor that may be present in both leaves and stems. This factor might bind to this 20-bp sequence (−242 to −223) to mediate the wound signal. The −242/−236 region contained a 7-bp sequence (TAACAAT) that is similar to TAACAAA box, which is recognized by HvGAMYB protein, a putative transcriptional activator of several GA-regulated genes (Gubler et al., 1995, 1999). Numerous MYB genes have been identified in higher plants. For example, Arabidopsis is estimated to contain more than a hundred MYB genes (Martin and Pazares, 1997), and a variety of these plant MYB proteins are considered to be involved in the control of many cellular responses to environmental stimuli, such as drought conditions and pathogen infection (Urao et al.,

1993; Yang and Klessig, 1996). Thus, the 7-bp sequence in the *FAD7* promoter might function as a *cis*-acting element that is recognized by unidentified wound-responsive plant MYB proteins. Previous studies of the wound-responsive nuclear factors in potato plants have delineated the leaf nuclear factors that interact with the promoter fragment of proteinase inhibitor II (*pinII*), but the results are controversial. Sánchez-Serrano et al. (1990) showed that mechanical wounding of leaves had no effect on binding activity of nuclear proteins to this promoter fragment. In contrast, Palm et al. (1990) reported a wound-inducible nuclear protein interacted with the *pinII* promoter.

The 60-bp fragment (−262/−203) failed to direct wound induction when it was fused to a cauliflower mosaic virus 35S minimal promoter. Thus, we cannot rule out the possibility that loss of wound responsiveness of the *FAD7* promoter with deletion of the −242/−223 region might reflect a change in the organization of functional elements in the *FAD7* promoter. However, since a wound-inducible factor bound to the −242/−223 region, this site is a strong candidate for a wound-responsive element. One possibility is that both other *cis*-acting elements and the 20-bp sequence may be required for complete wound response mediated by the *FAD7* promoter. In fact, deletion of the −202/−76 region from the −259 promoter abolished the wound responsiveness in leaves and stems (data not shown). We also found several nuclear factors interacting specifically with the −202/−76 region of the *FAD7* promoter in both leaf and stem nuclear extracts, although their binding activities were not significantly regulated by the wound signal (data not shown). Further analysis will be necessary to establish the role of these regions in wound response of the *FAD7* gene in the aerial part of the plant.

The wound responsiveness of the *FAD7* promoter in leaves was apparently increased by removal of the −825/−521 region (Fig. 1). In addition, gradual decrease of the response of this promoter to wounding were observed in stems when this promoter fragment was deleted stepwise to −259 (data not shown). Therefore, although we mapped the −259/−198 region as a wound-responsive domain in leaves and stems, some *cis*-elements in other regions of the *FAD7* promoter might be involved in wound responsiveness of this promoter. Furthermore, like photosynthetic genes, exogenous application of MeJA reduced the *FAD7* promoter activity in leaves of the −825 promoter-*GUS* plants (Reinbothe et al., 1994; Nishiuchi et al., 1997). These suggest that a complicated mechanism regulates the expression of the *FAD7* gene in leaves and stems. Therefore, our identification of a wound-responsive region in both leaves and stems could be the first and important step in delineating this complicated mechanism.

Our previous study suggested that the *FAD7* promoter activity in roots is modulated by JA biosynthesis in response to wounding (Nishiuchi et al., 1997). We showed that both wound- and JA-responsive elements in roots were mapped to the same region (−521 to −363) of the *FAD7* promoter. Two tobacco nuclear factors from the root bound to the −433/−363 sequence and showed different properties. Formation of complex R1, shown in Figure 6A, was induced more clearly by MeJA treatment than by wound treatment, and can therefore be termed the JA-inducible factor. When roots are wounded, this JA-inducible factor should be activated as a result of wound-induced JA accumulation.

The level of induction caused by MeJA application alone was equivalent to that caused by wound treatment (Figs. 1 and 2). In this case, the binding activity of the JA-inducible factor was much stronger than that in wounded roots (Fig. 6A), probably due to the relatively high concentration of MeJA to which the plants were subjected (Nishiuchi et al., 1997). In MeJA-treated roots, induction by the *FAD7* promoter may be mediated by the binding of the JA-inducible factor alone. The −433/−363 region of the *FAD7* promoter contains a sequence (CACTTG) that is similar to the G-box motif (CACGTG). G-box-like motifs were also found in the MeJA-responsive domains in the promoter regions of the bean *vsp* gene (Mason et al., 1993) and the potato *pinII* gene (Kim et al., 1992). The G-box motif is known to be the binding site of basic Leu zipper (bZIP) proteins (Menkens et al., 1995), but to our knowledge, no bZIP factors that specifically interact with MeJA-responsive domains have been identified. In fact, Williams et al. (1992) determined the nucleotide sequences of the bZIP binding sites and found that the G-box-like motif in the *FAD7* promoter is not likely to be recognized by bZIP proteins. An alternative possibility is that both G-box and G-box-like motifs are the putative binding sites (CANNTG) of basic helix-loop-helix (bHLH) proteins (Kawagoe and Murai, 1996; Rushton and Somssich, 1998). Identification of the JA-inducible factor and its precise binding site would be helpful for understanding the JA-dose-dependent induction in roots.

The nuclear factor in the complex R2 shown in Figure 6A was found in the wounded roots, but not in the untreated or the MeJA-treated roots. Therefore, the binding activity of this wound-inducible factor may be regulated independent of the octadecanoid pathway. Despite the fact that the DNA binding activity of this factor is specific to wounded roots, this factor alone was not sufficient for the transcriptional activation of the *FAD7* promoter in roots, since prefeeding with inhibitors of JA biosynthesis effectively suppressed wound activation by the *FAD7* promoter in roots (Nishiuchi et al., 1997). Thus, in wounded roots, transcriptional activation by the *FAD7* promoter might be controlled by the cooperative action of JA- and wound-inducible factors. Although this hypothesis should be examined by further analyses, the cooperative action among several transcriptional factors has been reported previously (Abe et al., 1997; Martin and Pazares, 1997; for review, see Yanagisawa, 1998).

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LITERATURE CITED

Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell **9:** 1859–1868

Bowles DJ (1990) Defense-related proteins in higher plants. Annu Rev Biochem **59:** 873–907

Creelman RA, Tierney ML, Mullet JE (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls

and modulate wound gene expression. Proc Natl Acad Sci USA **89:** 4938–4941

Farmer EE (1994) Fatty acid signalling in plants and their associated microorganisms. Plant Mol Biol **26:** 1423–1437

Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell **4:** 129–134

Farmer EE, Weber H, Vollenweider S (1998) Fatty acid signaling in *Arabidopsis*. Planta **206:** 167–174

Green PJ, Kay SA, Lam E, Chua N-H (1989) In vitro DNA footprinting. *In* SB Gelvin, RA Schilperoort, DPS Verma, eds, Plant Molecular Biology Manual, Vol B11. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–22

Gubler F, Kalla R, Roberts JK, Jacobsen JV (1995) Gibberellin-regulated expression of a *myb* gene in barley aleurone cells: evidence for Myb transactivation of a high-pI α-amylase gene promoter. Plant Cell **7:** 1879–1891

Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen JV (1999) Target genes and regulatory domains of the GAMYB transcriptional activator in cereal aleurone. Plant J **17:** 1–9

Hamada T, Kodama H, Nishimura M, Iba K (1994) Cloning of a cDNA encoding tobacco ω-3 fatty acid desaturase. Gene **147:** 293–294

Hamada T, Nishiuchi T, Kodama H, Nishimura M, Iba K (1996) cDNA cloning of a wounding-inducible gene encoding a plastid ω-3 fatty acid desaturase from tobacco. Plant Cell Physiol **37:** 606–611

Jupin I, Chua N-H (1996) Activation of the CaMV *as-1 cis*-element by salicylic acid: differential DNA-binding of a factor related to TGA1a. EMBO J **15:** 5679–5689

Kawagoe Y, Murai N (1996) A novel basic region/helix-loop-helix protein binds to a G-box motif CACGTG of the bean seed storage protein β-*phaseolin* gene. Plant Sci **116:** 47–57

Kim S-R, Choi J-L, Costa MA, An G (1992) Identification of G-box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor II promoter. Plant Physiol **99:** 627–631

Martin C, Pazares J (1997) MYB transcription factors in plants. Trends Genet **13:** 67–73

Mason HS, DeWald DB, Mullet JE (1993) Identification of a methyl jasmonate-responsive domain in the soybean *vspB* promoter. Plant Cell **5:** 241–251

McConn M, Creelman RA, Bell E, Mullet JE, Browse J (1997) Jasmonate is essential for insect defense in *Arabidopsis*. Proc Natl Acad Sci USA **94:** 5473–5477

Menkens AE, Schindler U, Cashmore AR (1995) The G-box: a ubiquitous regulatory DNA element in plants bound by the GBF family of bZIP proteins. Trends Biochem Sci **20:** 506–510

Nishiuchi T, Hamada T, Kodama H, Iba K (1997) Wounding changes the spatial expression pattern of the Arabidopsis plastid ω-3 fatty acid desaturase gene (*FAD7*) through different signal transduction pathways. Plant Cell **9:** 1701–1712

Nishiuchi T, Iba K (1998) Roles of the plastid ω-3 fatty acid desaturase in defense response. J Plant Res **111:** 481–486

Nishiuchi T, Nakamura T, Abe T, Kodama H, Nishimura M, Iba K (1995) Tissue-specific and light-responsive regulation of the promoter region of the *Arabidopsis thaliana* chloroplast ω-3 fatty acid desaturase gene (*FAD7*). Plant Mol Biol **29:** 599–609

O'Donnell PJ, Truesdale MR, Calvert CM, Dorans A, Roberts MR, Bowles DJ (1998) A novel tomato gene that rapidly responds to wound- and pathogen-related signals. Plant J **14:** 137–142

Palm CJ, Costa MA, An G, Ryan CA (1990) Wound-inducible nuclear protein binds DNA fragments that regulate a proteinase inhibitor II gene from potato. Proc Natl Acad Sci USA **87:** 603–607

Peña-Cortés H, Albrecht T, Prat S, Weiler EW, Willmitzer L (1993) Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta **191:** 123–128

Reinbothe S, Mollenhauer B, Reinbothe C (1994) JIPs and RIPs: the regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. Plant Cell **6:** 1197–1209

Rushton PJ, Somssich IE (1998) Transcriptional control of plant genes responsive to pathogens. Curr Opin Plant Biol **1:** 311–315

Sánchez-Serrano JJ, Peña-Cortés H, Willmitzer L, Prat S (1990) Identification of potato nuclear proteins binding to the distal promoter region of the proteinase inhibitor II gene. Proc Natl Acad Sci USA **87:** 7205–7209

Suzuki K, Suzuki N, Ohme-Takagi M, Shinshi H (1998) Immediate early induction of mRNAs for ethylene-responsive transcription factors in tobacco leaf strips after cutting. Plant J **15:** 657–665

Titarenko E, Rojo E, León J, Sánchez-Serrano JJ (1997) Jasmonic acid-dependent and -independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. Plant Physiol **115:** 817–826

Urao T, Yamaguchi-Shinozaki K, Urao S, Shinozaki K (1993) An Arabidopsis *myb* homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell **5:** 1529–1539

Weber H, Vick BA, Farmer EE (1997) Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family. Proc Natl Acad Sci USA **94:** 10473–10478

Williams ME, Foster R, Chua N-H (1992) Sequences flanking the hexameric G-box core CACGTG affect the specificity of protein binding. Plant Cell **4:** 485–496

Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Pérez-Grau L, Kinney AJ, Hitz WD, Booth JR Jr, Schweiger B, Stecca KL, Allen SM, Blackwell M, Reiter RS, Carlson TJ, Russell SH, Feldmann KA, Pierce J, Browse J (1993) Cloning of higher plant ω-3 fatty acid desaturases. Plant Physiol **103:** 467–476

Yanagisawa S (1998) Transcriptional factor in plants: physiological functions and regulation of expression. J Plant Res **111:** 363–371

Yang Y, Klessig DF (1996) Isolation and characterization of a tobacco mosaic virus-inducible *myb* oncogene homolog from tobacco. Proc Natl Acad Sci USA **93:** 14972–14977